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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/595, 7/06, A61K 39/00, 39/39		A2	(11) International Publication Number: WO 95/13297
			(43) International Publication Date: 18 May 1995 (18.05.95)
(21) International Application Number: PCT/US94/13205			(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, NL, NO, NZ, PL, PT, RO, RU, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).
(22) International Filing Date: 10 November 1994 (10.11.94)			
(30) Priority Data: 08/151,219 12 November 1993 (12.11.93) US			
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(74) Agents: DRIVAS, Dimitrios, T. et al.; White & Case, Patent Dept., 1155 Avenue of the Americas, New York, NY 10036 (US).			Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: IMPROVED IMMUNOGENIC COMPOSITIONS AGAINST HUMAN GASTRIN 17			
(57) Abstract <p>An improved immunogenic composition against human gastrin 17 comprising the peptide pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Ser-Ser-Pro-Pro-Pro-Cys (SEQ ID NO.:1) coupled to an immunogenic carrier and pharmaceutical compositions containing the same.</p>			

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IMPROVED IMMUNOGENIC COMPOSITIONS AGAINST HUMAN GASTRIN 17

5 Background and Summary of the Invention

Immunization against specific disease promoting hormones may be useful in the treatment and prevention of certain diseases and cancers. Such immunological approaches to the treatment and prevention of gastric and duodenal ulcer disease and gastro-intestinal cancer are disclosed in co-assigned U.S. Patent Number 5,023,077 and PCT application WO 90/08774. According to these immunological approaches, specific antibodies neutralize the biological activity of disease promoting gastrointestinal peptide hormones. The antibodies are specific for a particular hormone, and one or more hormones can be selectively targeted to treat a particular disease. For example, human gastrointestinal hormone gastrin 17 ("hG17") is involved in gastrointestinal disease processes including gastro-esophageal reflux disease, gastric and duodenal ulceration and cancer. Specific anti-hG17 antibodies which neutralize the action of hG17 can therefore be used to treat diseases in which hG17 is involved. The anti-hormone antibodies can be administered to the patient (i.e., passive immunization) or they can be induced in the patient by active immunization.

Active immunization against gastrointestinal peptide hormones is accomplished by administering to the patient an immunogen that contains chemical structures that induce antibodies which bind to the targeted hormone. Such chemical structures are defined as immunomimics of the targeted hormone, and can be composed of any molecule that immunologically crossreacts with the targeted hormones. Immunomimics may inherently possess the capacity to induce antibodies, e.g., they may be immunogenic, however, frequently, immunomimics are not inherently immunogenic, and they must be linked to immunogenic carrier molecules to be rendered immunogenic.

The immunogens of U.S. Patent Number 5,023,077, the disclosure of which is hereby incorporated by reference in its entirety, and of the present invention comprise an immunogenic carrier, such as diphtheria toxoid ("DT"), to which is

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Brief Description of the Figures

FIGURE 1 depicts antibody responses in rabbits as measured by mean antibody binding capacity ("ABC") in pico moles per ml. in response to three immunizations with immunogens comprising each of the conjugates hG17(1-9)-Ser9-DT; hG17(1-9)-Arg9-DT; and hG17(1-6)-Arg6-DT.

FIGURE 2 depicts the antibody response in rabbits as measured by mean antibody binding capacity ("ABC") in pico moles per ml immunized with one administration of a conjugate constructed with peptide 1 (hG17(1-9)-Ser9-DT) of Example 1.

Detailed Description of the Invention**EXAMPLE 1**

Peptides were prepared by standard solid state synthesis methods. Each peptide was characterized as to amino acid content and purity.

Peptides with the following amino acid sequences were synthesized:

<u>Peptide</u>	<u>Designation</u>	<u>Amino Acid Sequence</u>
1	hG17(1-9)-Ser9	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Ser-Ser-Pro-Pro-Pro-Pro-Cys (SEQ ID NO.: 1)
2	hG17(1-9)-Arg9	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Arg-Pro-Pro-Pro-Pro-Cys, identified as SEQ ID NO.: 4 in the Sequence Listing)

Each of Peptides 1-2 contains an amino terminal immunomimic of hG17 followed by a carboxy terminal spacer. Peptide 1 comprises a 9 amino acid immunomimic of hG17 (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-, SEQ ID NO.:2) followed by the "Ser" spacer (-Ser-Ser-Pro-Pro-Pro-Pro-Cys, SEQ ID NO.:3) attached to amino acid number 9 of the hG17 immunomimic. Peptide 2 comprises the 9 amino

acid immunomimic (the same as in Peptide 1) followed by the "Arg" spacer (-Arg-Pro-Pro-Pro-Cys, identified as SEQ ID NO.: 5 in the Sequence Listing) as described in U.S. Patent No. 5,023,077.

5 Each of these peptides was conjugated to amino groups present on the Diphtheria Toxoid ("DT") immunogenic carrier via the terminal peptide cysteine residue utilizing heterobifunctional linking agents containing a succinimidyl ester at one end and maleimide at the other end of the linking agent essentially as described in U.S. Patent No. 5,023,077. To accomplish the linkage between either of the
10 Peptides 1-2 above and the carrier, the cysteine of the peptide was first reduced. The dry peptide was dissolved in 0.1 M sodium phosphate buffer, pH 8.0 with a thirty molar excess of dithiothreitol. The solution was stirred under a water saturated nitrogen gas atmosphere for four hours at room temperature. The peptide containing reduced cysteine was separated from the other components by chromatography at 4°C
15 over a G10 Sephadex column equilibrated with 0.2 M acetic acid. The peptide was lyophilized and stored under vacuum until used.

 The DT was activated by treatment with the heterobifunctional linking agent epsilon-maleimidocaproic acid N-hydroxysuccinimide ester ("EMCS"), in proportions
20 sufficient to achieve activation of approximately 25 free amino groups per 10⁵ molecular weight of DT. In the specific instance of DT, this amounted to the addition of 6.18 mg of EMCS (purity 75%) to each 20 mg of DT.

 The DT was activated by dissolving 20 mg of DT in 1 ml of 0.5 M sodium
25 phosphate buffer, pH 6.6. Separately 6.18 mg EMCS were dissolved into 0.2 ml of dimethyl formamide. Under darkened conditions, the EMCS was added dropwise in 50 microliter ("μl") amounts to the DT with stirring. After 2 hours of incubation at room temperature in darkness, the mixture was chromatographed at 4°C on a G50 Sephadex column equilibrated with 0.1 M sodium citrate buffer, pH 6.0, containing
30 0.1 mM ethylenediaminetetraacetic acid disodium salt ("EDTA").

 Fractions containing the EMCS activated DT were pressure concentrated over a PM 10 ultrafiltration membrane under nitrogen gas in conditions of darkness. The

protein content of the concentrate was determined by the BCA method (PIERCE, IL, USA). The EMCS content of the carrier was determined by incubation of the activated DT with cysteine-HCl followed by reaction with 100 μ l of 10 mM Elman's Reagent (5'5'dithio-bis (2-nitrobenzoic acid)). The optical density difference between
5 a blank tube containing cysteine-HCl and the sample tube containing cysteine-HCl and carrier was translated into 25 EMCS group content by using the molecular extinction coefficient of 13.6×10^3 for 5-thio-2-nitro-benzoic acid at 412 nm.

The reduced cysteine content ("SH") of the peptide was also determined
10 utilizing Elman's Reagent. Approximately 1 mg of peptide was dissolved in 1 ml of nitrogen gas saturated water and a 0.1 ml aliquot of this solution was reacted with Elman's Reagent. Utilizing the molar extinction coefficient of 5-thio-2-nitro-benzoic acid (13.6×10^3), the free cysteine -SH was calculated.

15 To conjugate the reduced peptide to the activated DT, an amount of peptide containing sufficient free -SH to react with each of the EMCS activated amino groups on the DT was dissolved in 0.1 M sodium citrate buffer, pH 6.0, containing 0.1 mM EDTA, and added dropwise to the EMCS activated DT under darkened conditions. After all the peptide solution had been added to the activated DT, the mixture was
20 incubated overnight in the dark under a water saturated nitrogen gas atmosphere at room temperature.

The conjugate of the peptide linked to DT via EMCS was separated from other components of the mixture by low pressure chromatography at 4°C over a G50
25 Sephadex column equilibrated with 0.2 M ammonium bicarbonate. The conjugate eluted in the column void volume and was lyophilized and stored desiccated at 20°C until used.

The conjugate may be characterized as to immunomimic peptide content by a
30 number of methods known to those skilled in the art including weight gain, amino acid analysis, etc. Conjugates of Peptides 1-2 to DT produced by these methods were determined by amino acid analysis to have 15-28 moles of peptide per 10^5 MW of DT and all were considered suitable as immunogens for immunization of test animals.

EXAMPLE 2

The peptide-DT conjugates of Example 1 were administered in emulsions of aqueous and oily phase components that were prepared as follows. The conjugate and nor-MDP adjuvant were dissolved in phosphate buffered saline ("PBS") to produce the aqueous phase. The aqueous phase is prepared so that the concentrations of conjugate and nor-MDP are double the concentration that these components will have in the final emulsion. In order to prepare the immunogens used in Example 4, the conjugate was dissolved in phosphate buffered saline ("PBS"), pH=7.2, to a concentration of 8.0 mg/ml. Nor-MDP adjuvant was dissolved in PBS to a concentration of 0.4 mg/ml. These two PBS solutions were then mixed together in a 1:1 ratio (vol:vol), yielding an aqueous phase solution containing 4.0 mg/ml conjugate and 0.2 mg/ml nor-MDP.

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The aqueous phase was combined 1:1 (vol:vol) with the oily vehicle phase to create an emulsion that comprised the final immunogen formulation. Various types of oily vehicles, known to those skilled in the art, may be used. One such vehicle is a mixture of four parts squalene and one part arlacel. The preferred oily vehicle for use with the immunogens of the invention is stabilized Montanide ISA 703 produced by Seppic (Paris, France). Montanide ISA 703 is not satisfactory for use alone and must have a stabilizing component added to it so that it can be used in the emulsion. The aqueous phase and oily phase vehicle can be mixed by any method known to those skilled in the art to form a stable emulsified mixture. The emulsion must be stable upon storage (e.g., not undergo a significant degree of separation into aqueous and vehicle phases for a minimal storage time of several weeks) and it must be of a consistency that allows it to be easily injected through an acceptable size hypodermic needle.

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To stabilize Montanide ISA 703 oily vehicle we added aluminum monostearate ("AMS"). To determine the correct concentration of AMS, various concentrations of AMS were tested with the Montanide ISA 703 vehicle. The AMS was USP/NF Grade, #AL228, from Spectrum Chemical Manufacturing Corp. (Gardena, California,

USA). Samples of Montanide ISA 703 were tested to which the following percent concentrations of AMS were added: 0; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8; 2.0; 2.4; and 2.8%. Emulsions were made with an aqueous phase containing 4.0 mg/ml of conjugate and 0.5 mg/ml of nor-MDP adjuvant as described below with each sample vehicle preparation and assessed for stability and viscosity. An AMS range from about 1.5% to about 20% W/W was found to be acceptable. Montanide ISA 703 containing 1.6% and 1.8% AMS produced satisfactory emulsions, with 1.8% AMS being preferable. The sample vehicle preparations containing percent concentrations of 2.0% AMS and above produced emulsions that were too viscous and the sample vehicle containing 1.4% or less AMS produced unstable emulsions or totally failed to emulsify. The vehicle used in this application for administering the immunogens was Montanide ISA 703 containing 1.8% AMS, and is referred to as "Montanide ISA 703 AMS."

The aqueous phase containing the immunogen was emulsified 1:1 (vol:vol) with the Montanide ISA 703 AMS vehicle phase by pressing a mixture of the two solutions through an 18 gauge double hubbed needle between two glass syringes. The mixture was pressed through the needle 40 times. The emulsified mixture was then drawn into disposable syringes for injection into animals. The final concentrations in the emulsion of the immunogens used in Example 4 were: conjugate=hG17(1-9)Ser9-DT:2.0 mg/ml.; nor-MDP adjuvant:0.25mg/ml. The concentration of AMS in the oily vehicle was 1.8% which resulted in 0.9% AMS in the final mixed emulsion.

EXAMPLE 3

We constructed conjugates comprising each of the peptides listed in Example 1 linked to DT, as described in Example 1 and 2. We then immunized rabbits. Ten Rabbits were immunized with the Peptide 1 immunogen, and four rabbits with the Peptide 2 immunogen. We additionally immunized four rabbits with hG17(1-6)-Arg6 linked to DT as set forth in U.S. Patent No. 5,023,077. The conjugates were administered in emulsions prepared as in Example 2, except that the oily vehicle phase consisted of a squalene:arlacel solution (comprising 4 parts squalene to 1 part

arlacel) and the final concentrations of conjugate were 1.0 mg/ml and adjuvant were 0.2 mg/ml in each emulsion. A 0.5 ml. aliquot of the emulsion was injected into each rabbit. Each rabbit was given immunizations on days 0, 21 and 42 of the tests with 0.5 mg of conjugate injected intramuscularly per dose. Blood was collected
5 from each rabbit prior to the first injection (day 0) and on days 14,35,56 and 84. Serum was prepared from each blood sample and stored at -20°C until utilized in assays to determine the presence of anti-gastrin antibodies. Anti-hG17 antibody levels were determined by RIA.

A liquid phase Radioimmunoassay (RIA) was used to detect and quantify anti-gastrin antibodies. In the RIA, 1.0 or 10.0 μ l aliquots of antiserum were incubated
10 with approximately 250 pg of 125 I labeled hG17 (specific activity=18 Ci/m mole) total volume of 400 μ l. Dilutions were made in FTA Hemagglutination Buffer (BBL, Becton Dickinson Microbiology Systems, MD, USA) containing 1% bovine serum albumin. The antisera were incubated with labeled hormone overnight at 4°C.
15 Following this incubation, 0.1 ml of heat inactivated (56°C, 30 min) fetal calf serum at 4°C was then added to each tube. The antibody-hormone complexes were then precipitated by the addition of 0.1 ml of 25% polyethylene glycol (MW=8,000 gm/mole) at 4°C. The precipitates were pelleted by centrifugation (30 minutes at 1000 x g), the supernatants were discarded, and the pellets were counted with a
20 gamma counter to measure the quantity of radioactivity contained therein. Antigen binding capacities (ABC) for each antiserum were then determined from the amount of radioactive hormone in the precipitate. Serum taken from the rabbits prior to immunization served as nonimmunized (normal) controls. Nonspecific background binding was subtracted from all values. To demonstrate the specificity of the reaction
25 of 125 I labeled hormone with the antisera, aliquots of the antisera were preincubated in some tests with excess amounts of hG17 that was not labeled with 125 I, to inhibit binding of the antisera to the labeled hormone.

The results of this test are presented in Table 1 and in Figure 1. As can be
30 seen therein, Immunogens 1 and 2 (of Example 1) were superior to Immunogen 3, in terms of both the potency and the duration of the anti-hG17 antibody responses induced by the immunogens.

The improvements to the immunogen arise from modifications made to the immunomimic and spacer regions of the peptide. The peptides that comprise Immunogens 2 and 3 have identical Arg spacers, but Immunogen 2 is considerably more potent because its peptide has an improved immunomimic of hG17 (for day 84, $p=0.05$, Student's t test). Conversely, the peptides that comprise Immunogens 1 and 2 incorporate the same immunomimic of hG17; yet, Immunogen 1 is more immunogenic because it possesses a superior spacer element (the Ser spacer) (for day 84, $p=0.001$). The immunogens thus can be improved by modifying the spacer and/or the immunomimic.

TABLE 1
RABBIT SERUM ANTI-hG17 ANTIBODY LEVELS
INDUCED BY ANTI-hG17 IMMUNOGENS
ADMINISTERED ON DAYS 0, 21 AND 42

IMMUNOGEN NUMBER	PEPTIDE DESIGNATION	MEAN RIA ABC (+/- s.e.) [pmole/ml]				
		Day 0	Day 14	Day 35	Day 56	Day 84
1	hG17(1-9)-Ser9	0	1 +/- 1	31 +/- 4	117 +/- 6	170 +/- 8
2	hG17(1-9)-Arg9	0	1 +/- 1	25 +/- 7	98 +/- 22	76 +/- 18
3	hG17(1-6)-Arg6	0	0	10 +/- 2	23 +/- 2	11 +/- 3

EXAMPLE 4

Six female rabbits were immunized with the hG17(1-9)Ser9-DT conjugate produced by the methods of Example 1 and 2 by intramuscular administration. The immunogen comprised 2.0 mg/ml hG17(1-9)Ser9-DT conjugate and 0.25 mg/ml nor-MDP adjuvant in PBS emulsified with Montanide ISA 703 AMS. Each rabbit was injected only on day 0 of the test. The volume injected was 1.0 ml. per rabbit. Every 7 days thereafter, blood samples were obtained from each rabbit. Serum was prepared from each blood sample and stored at -20°C , until utilized in assays to determine the presence of anti-gastrin antibodies.

The mean ABCs measured in the sera from rabbits immunized with the immunogens of Example 1 are shown in Table 2 and in Figure 2. As these results show, a single administration of immunogen induced a rapid and potent antibody response against hG17. 42 days after the immunogen was injected, a mean antibody level of 227 pmoles of hG17 bound per ml of antiserum had been induced in the rabbits. As can be seen in Figure 2, the anti-hG17 antibody response was still increasing at a rapid rate on day 42.

TABLE 2
RABBIT SERUM ANTI-hG17 ANTIBODY LEVELS
INDUCED BY IMMUNIZATION
ON DAY 0 OF THE TEST

Mean RIA ABC (\pm s.e.) [pmole/ml]							
Day of Test	0	7	14	21	28	35	42
ABC	0	3 \pm 1	22 \pm 6	82 \pm 20	138 \pm 27	197 \pm 30	227 \pm 25

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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10 (ii) TITLE OF INVENTION: IMPROVED IMMUNOGENIC COMPOSITIONS
 AGAINST HUMAN GASTRIN 17

(iii) NUMBER OF SEQUENCES: 5

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(v) COMPUTER READABLE FORM:

 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

 (A) APPLICATION NUMBER:
30 (B) FILING DATE: 12-NOV-1993
 (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

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(B) REGISTRATION NUMBER: 32,218

(C) REFERENCE/DOCKET NUMBER: 1102865-028

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(2) INFORMATION FOR SEQ ID NO:1:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Glu Gly Pro Trp Leu Glu Glu Glu Glu Ser Ser Pro Pro Pro Pro Cys

1

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10

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5

Glu Gly Pro Trp Leu Glu Glu Glu Glu

1 5

(2) INFORMATION FOR SEQ ID NO:3:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

20

Ser Ser Pro Pro Pro Pro Cys

1 5

(2) INFORMATION FOR SEQ ID NO:4:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

CLAIMS

We claim :

- 5 1. A immunogenic composition comprising the peptide pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Ser-Ser-Pro-Pro-Pro-Cys SEQ ID NO.: 1, coupled to an immunogenic carrier.
2. The immunogenic composition of claim 1 wherein the immunogenic
10 carrier is selected from the group consisting of diphtheria toxoid, tetanus toxoid and keyhole limpet hemocyanin.
3. The immunogenic composition of claim 2 wherein the immunogenic carrier is diphtheria toxoid.
15
4. A pharmaceutical composition comprising an effective amount of the immunogenic composition of claims 1, 2 or 3 and a pharmaceutically acceptable carrier.
- 20 5. The pharmaceutical composition of claim 4 wherein the pharmaceutically acceptable carrier comprises an emulsion of an aqueous phase and an oily phase, wherein the oily phase is an oily vehicle comprising Montanide ISA 703 containing from about 1.5% W/W to about 2.0% W/W aluminum monostearate.
25
6. The peptide pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Ser-Ser-Pro-Pro-Pro-Pro-Cys, SEQ ID NO.: 1.
7. The spacer peptide -Ser-Ser-Pro-Pro-Pro-Pro-Cys, SEQ ID NO.: 3.
30

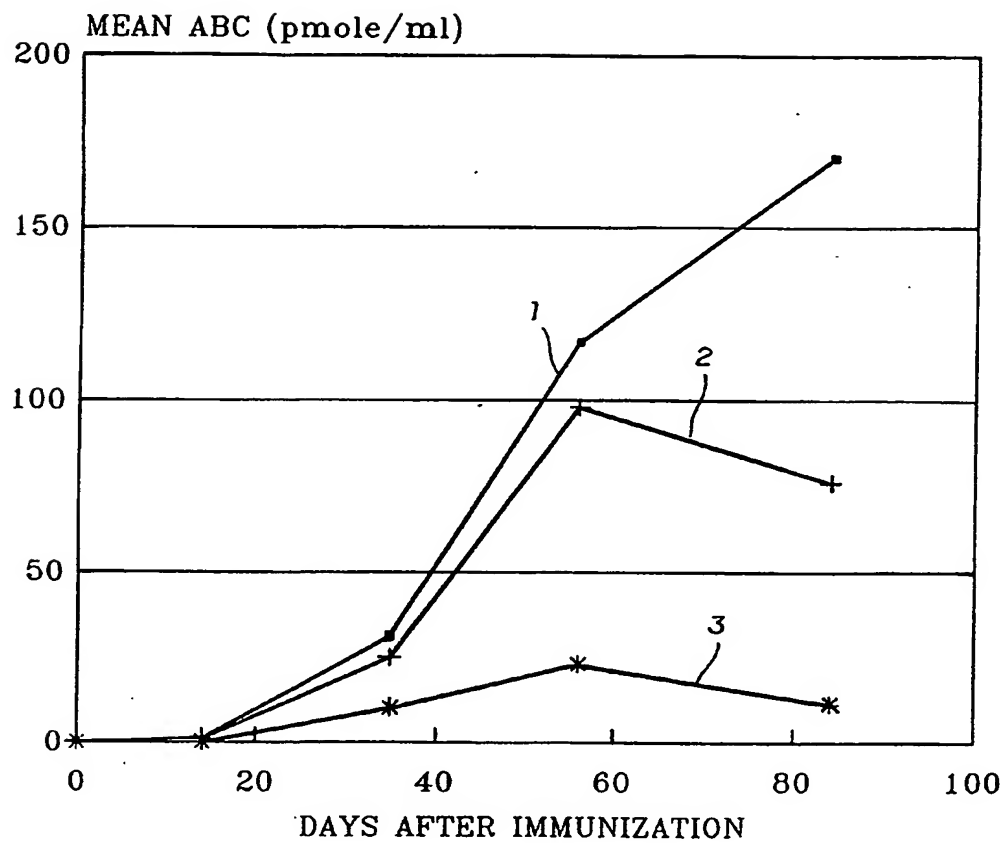


FIG. 1

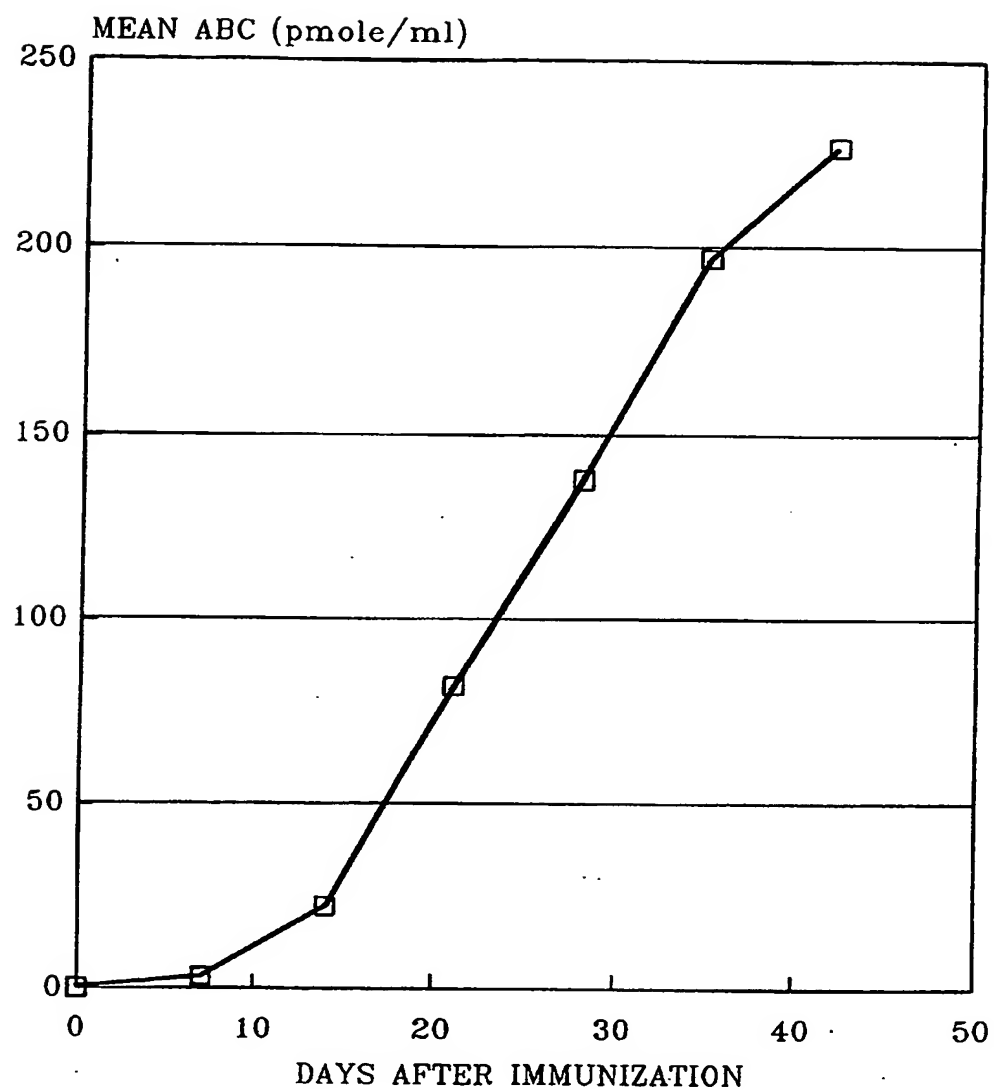


FIG. 2

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/595, A61K 39/00, 39/39	A3	(11) International Publication Number: WO 95/13297 (43) International Publication Date: 18 May 1995 (18.05.95)
(21) International Application Number: PCT/US94/13205 (22) International Filing Date: 10 November 1994 (10.11.94) (30) Priority Data: 08/151,219 12 November 1993 (12.11.93) US (71) Applicant: APHTON CORP. [US/US]; World Trade Center Miami, Suite 2160, 80 S.W. 8th Street, Miami, FL 33130 (US). (72) Inventors: GEVAS, Philip, C.; 487-A Portlock Road, Honolulu, HI 96825 (US). GRIMES, Stephen; 551 Rutgers Drive, Davis, CA 95616 (US). KARR, Stephen, L.; 624 Villanova Drive, Davis, CA 95616 (US). MICHAELI, Dov; 21 Marina Vista Avenue, Larkspur, CA 94939 (US). SCIBI-ENSKI, Robert; 803 College Street, Woodland, CA 95776 (US). (74) Agents: DRIVAS, Dimitrios, T. et al.; White & Case, Patent Dept., 1155 Avenue of the Americas, New York, NY 10036 (US).	(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, NL, NO, NZ, PL, PT, RO, RU, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 15 June 1995 (15.06.95)	
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ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/13205

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/595 A61K39/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 380 230 (APHTON CORPORATION) 1 August 1990 cited in the application see the whole document ---	1-7
P,A	GASTROENTEROLOGY, vol. 106,no. 4, April 1994 page A824 R.MAKISHIMA ET AL 'Active immunisation against gastrin-17 with an N-terminal derived immunogen inhibits gastric and duodenal lesions in rats' see the whole document -----	1-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 April 1995

Date of mailing of the international search report

10.05.95

Name and mailing address of the ISA

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Authorized officer

Groenendijk, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/13205

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-380230	01-08-90	US-A- 5023077	11-06-91
		AT-T- 114160	15-12-94
		AU-B- 645967	03-02-94
		AU-A- 5082090	24-08-90
		DE-D- 69014137	22-12-94
		ES-T- 2063912	16-01-95
		JP-T- 4503072	04-06-92
		WO-A- 9008774	09-08-90
